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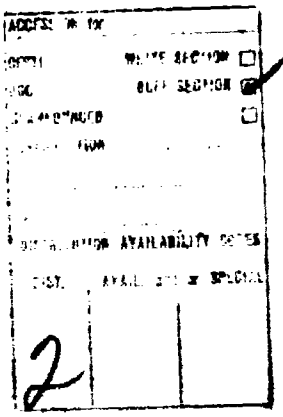
TECHNICAL MANUSCRIPT 604

DIRECT ASSESSMENT OF VIRAL AEROSOLS ON CELL CULTURES

William A. Hankins
Henry J. Hearn

MAY 1970

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland



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TECHNICAL MANUSCRIPT 604

DIRECT ASSESSMENT OF VIRAL AEROSOLS ON CELL CULTURES

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Experimental Aerobiology Division
AEROBIOLOGY & EVALUATION LABORATORIES

Project 1B562602AD03

May 1970

DIRECT ASSESSMENT OF VIRAL AEROSOLS ON CELL CULTURES*

ABSTRACT

A technique is described for the direct exposure of cell cultures to airborne virus, enabling quantitation of the virus in concentrations as low as one plaque-forming unit per liter of air.

Methods of sampling airborne microorganisms, particularly viruses, usually require their impingement in a suitable collection fluid, although techniques may include collection on agar, on a filter, or by electrostatic precipitation.[1] The collected viral agent must then be quantitated by conventional assay procedures, i.e., by inoculation into laboratory animals, embryonated eggs, or cell cultures. This report describes a technique in which known volumes of viral aerosols were drawn directly into flasks containing cell culture monolayers that were then used to quantitate the virus by allowing plaques to develop. This technique permits the detection of as low as one plaque-forming unit (PFU) of airborne virus per liter of air.

Venezuelan equine encephalomyelitis (VEE) virus, Trinidad strain grown in embryonated eggs was selected for study. Viral aerosols were generated in a cylindrical, 1,200-liter chamber similar to that described by Wolfe,[2] using either an FK-8 gun[3] or a Collison device.[4] Monolayers of primary chicken embryo (CE) cells or an established line of guinea pig lung cells were prepared in 250-ml cell culture flasks,** using Eagle's basal medium supplemented with 10% fetal calf serum, 100 units of penicillin, and 100 μg of streptomycin per ml. The CE cells were subsequently shown to be more uniformly susceptible to the virus and were chosen for most of the experimental work.

Cell cultures drained of growth media were exposed to the viral aerosols by attaching the mouth of the flask to the chamber. Before attaching the flask to the chamber, a hole was burned through the opposite end of the flask with a hot metal rod; a small sterile skirted stopper was inserted in this hole. A 15-gauge needle attached to a vacuum source with rubber tubing was used to penetrate the rubber stopper and to draw the aerosol into the flask. The rate of flow was controlled by a critical orifice inserted into the rubber tubing. When flasks were connected in series, this was accomplished by substituting metal caps containing 15-gauge needles and rubber gaskets for the plastic caps (Fig. 1).

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** Falcon Plastics, Los Angeles, California.

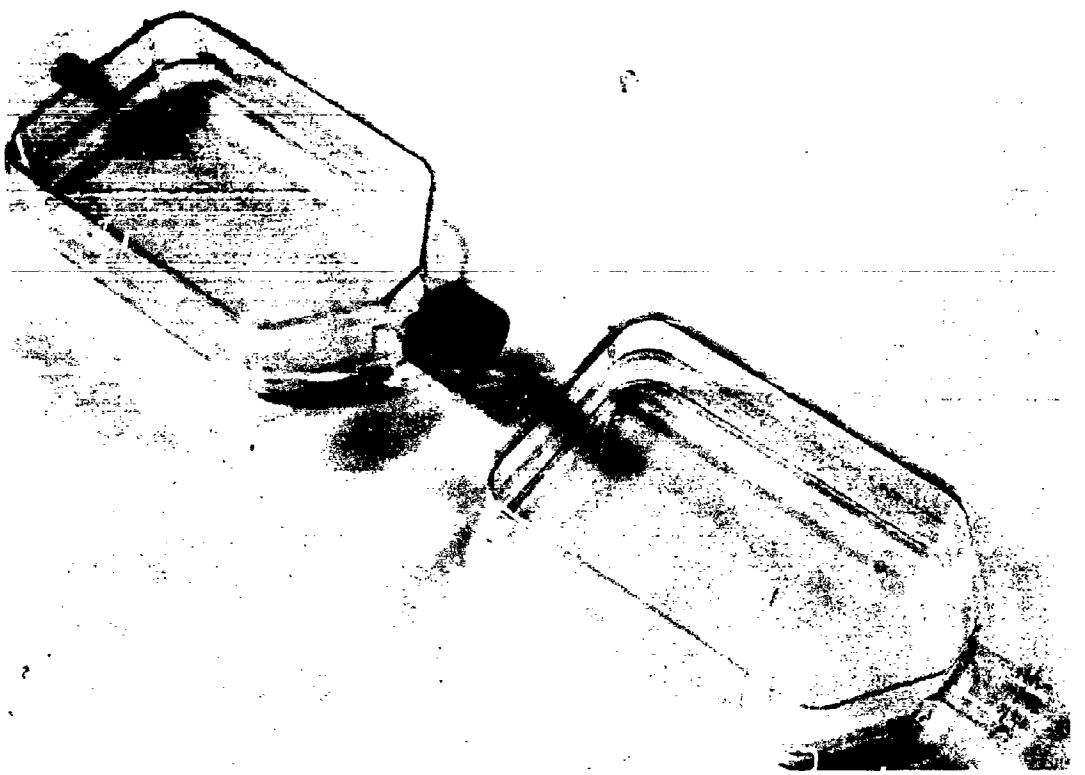

FIGURE 1. Cell Culture Flasks Used for Direct Assessment of Viral Aerosols.

After exposure to aerosols, cell monolayers were overlayed with Eagle's basal medium supplemented with 5% fetal calf serum, 1% agar, 100 units of penicillin, and 100 μg of streptomycin per ml. The length of time after exposure before the overlay was applied did not appear to be critical, but should be completed within an hour. Infected cells were incubated for 24 hours at 37 C; a second overlay containing, in addition, 0.01% neutral red was applied; plaques were counted in another 24 hours.

Plaque counts were not diminished by an air wash of the flasks immediately after exposure to the aerosol, suggesting a rapid and irreversible attachment of the virus to the cells. Treatment of the exposed flask with specific antiserum reduced or eliminated plaque formation.

The titers shown in Table 1 are average values of at least two tests and represent values corrected for a uniform total volume sampled. When virus diluted 1/300 was disseminated, the concentration of aerosolized virus as determined by the direct flask (DF) technique was approximately 2.6×10^3 PFU per liter. Dilution of the virus in tenfold increments prior to aerosolization resulted in corresponding decreases in recoveries when the sampling rate was 1 liter per min. It is of interest that when the sampling rate was increased to 10 liters per min, the recovery titers decreased somewhat, suggesting that with this faster sampling rate some virus might have escaped from the flask. Titer values obtained by the impinger technique (Table 1) were comparable to those obtained by the DF technique in the same experiment. Aerosolized virus originating from suspension concentrations of 1/30,000 or greater, however, were not detected by conventional impinger techniques.

The results of experiments designed to test for virus escape from flasks exposed to aerosols of different concentrations are shown in Table 2. In these tests, the aerosols were drawn through flasks connected in series rather than into only one flask. Aerosol concentrations were reduced by a series of air dilutions (purges) of the chamber, and, after each purge, the virus titer of the aerosol was determined. Data indicate that about 10% of the virus bypassed one flask and was deposited in the next flask at all concentrations. Also included in Table 2 are data showing that the assessments of virus per liter of aerosol by the DF technique were approximately the same as obtained by assaying impinger samples by the plaque technique.

In summary, results of these tests indicate that the direct exposure of cell culture monolayers to viral aerosols (DF technique) provides a simple and highly sensitive technique for assessing infectious airborne viral particles. The DF technique permitted the recovery of virus in very low aerosol concentrations that could not be detected by conventional impinger techniques.

TABLE 1. SAMPLING RATES AND RECOVERIES OF DILUTED AEROSOLIZED VEE VIRUS USING DIRECT FLASK AND IMPINGER TECHNIQUES

Virus Dilution[a]	Liters per Min[b]	Time, min	Total Sample Volume,[c] liter	PFU per Liter	
				Direct Flask	Impinger[d]
1/300	1	0.1	0.1	2600	2600
1/3000	1	1.0	1.0	160	310
	1	0.1	0.1	420	NT[e]
	10	0.1	1.0	70	NT
1/30,000	1	10.0	10.0	11	NR[f]
	10	1.0	10.0	2	NR
1/300,000	1	10.0	10.0	1.2	NR

a. In spray device prior to aerosolization.
b. Liters per minute limited by critical orifice used to draw aerosol into flask.
c. Total volume of air that passed through flask.
d. Virus in impinger fluid assayed by plaque test.
e. Not tested.
f. No virus recovered by conventional impinger technique.

TABLE 2. SAMPLING OF VEE VIRAL AEROSOLS USING FLASKS[a] IN SERIES AND IMPINGER TECHNIQUES

Sampling[b]	Flask No. 1	2	3	4	5	Concn per Liter,[f] plaques	Concn per Liter,[g] plaques
Initial	C[d]	C	>750[e]	162	21	1.83×10^6	3.9×10^6
After Purge 1[c]	C	>800[e]	140	22	-	1.62×10^5	1.95×10^5
After Purge 2	>800[e]	95	3	-	-	9.80×10^3	2.38×10^4
After Purge 3	123	16	-	-	-	1.39×10^3	4.80×10^3

a. As used in direct flask technique (see text and Fig. 1).
b. 100 ml of aerosol sampled in 0.1 minute.
c. Virus concentration reduced (purged) by air dilution of chamber.
d. Clear; total cell destruction.
e. TNTC, only estimate of count was possible.
f. Assay values based upon countable plaques.
g. Assay values based upon plaque counts obtained from impinger fluids.

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